Saskatchewan
Agriculture, Food
and Rural
Revitalization

ADF

AGRICULTURE DEVELOPMENT FUND

FINAL REPORT

20030467

CONTROL OF FOLLICULAR DEVELOPMENT AND OVULATION FOR FIXED-TIME ARTIFICIAL INSEMINATION (AI) IN CATTLE (SRP) TRANSITION

Funded by:
The Agriculture Development Fund

April 2004
Prepared by: University of Saskatchewan

104-06117/

FINAL REPORT
July 22, 2003 to March 31, 2004
ADF Project # 20030467
MIDAS # 000268
University of Saskatchewan Account Number 7-74581

CONTROL OF FOLLICULAR DEVELOPMENT AND OVULATION FOR FIXED-TIME ARTIFICIAL INSEMINATION (AI) IN CATTLE (SRP TRANSITION)

Reuben J. Mapletoft
WCVM, University of Saskatchewan

Abstract/Summary
Artificial insemination (AI) has not been widely used by the beef industry, in large part, because of the cost, time and effort required for detection of heat. However, fixed-time AI, without heat detection, can make AI feasible. Results show how fixed-time AI can be accomplished through hormonal manipulation, and how protocols can be improved, or why certain protocols are not efficacious under certain circumstances. Finally, approaches to the resynchronization of artificially inseminated cattle have been investigated. Results clearly show that fixed-time AI followed by resynchronization and AI can result in high pregnancy rates to AI sires.

Executive Summary
Estrus synchronization and artificial insemination (AI) have not been widely used by cow-calf producers due to substantial inputs, including cost of consumables, the time and labor needed for treatments and estrus detection, and unpredictable results. However, it is a matter of record how AI has facilitated genetic improvement in the dairy industry. The overall objective of this research was to facilitate the use of AI in beef cattle in order to utilize improved sires, and implement emerging methods of genetic selection and reproductive biotechnology. Fixed-time AI (by appointment) has reduced labor by eliminating estrus detection, but up until now has resulted in substantially reduced pregnancy rates. Projects that further refined regimens, largely developed by the collaborating researchers, that have synchronized ovulation, resulting in high fertility to fixed-time AI, without the need for estrus detection, were proposed. As cost, efficacy and user friendliness will influence use of AI as a genetic tool, it was hypothesized that fixed-time AI with high fertility would increase the use of this valuable tool by cattle producers. However, protocols have often been complicated and costly, and hormone preparations used have largely been available only through prescription. A series of experiments were designed to simplify protocols and reduce costs, and to utilize pharmaceuticals that were readily available. Specifically, we proposed to confirm results suggesting that regimens for synchronization of follicle waves and ovulation could be incorporated into progestin- (CIDR or MGA) or prostaglandin- (PGF) based synchronization programs, and that "repeats" in a fixed-time AI scheme could be re-synchronized to facilitate re-insemination.

We were fortunate to be able to complete several important experiments over the past year, but one had to be left for the coming year in order to take advantage an unexpected opportunity.

This is not uncommon when producer herds are used for experimental studies; the producer must be comfortable with the approach used. Briefly, detailed ultrasound studies suggested that estradiol valerate is as efficacious as estradiol-17β or estradiol benzoate in synchronizing follicular wave emergence, providing the appropriate dose is given. This is important because several practitioners have estradiol valerate remaining in inventory from when they used Syncro Mate-B to synchronize estrus. However, ultrasound studies also showed that administration of estradiol late in the estrous cycle is problematic, and may result in a failure to synchronize follicular wave emergence which would result in asynchronous ovulation following progestin removal. This could explain why estrus is occasionally not tightly synchronized despite the use of an apparently proven protocol.

In an attempt to investigate new approaches to the resynchronization of "repeats" following fixed-time AI, ultrasound studies showed that progesterone, GnRH or estradiol administered at the time of progestin insertion on Day 13 would resynchronize follicular wave emergence, but in a field trial, none were shown to influence estrus synchrony or pregnancy rate at the resynchronized estrus. However, the administration of 0.5 mg ECP at the time of progestin removal tended to increase the numbers of animals showing estrus, without adversely affecting pregnancy rate to the fixed-time insemination. In addition, fertility was higher when a used CIDR was utilized to resynchronize return to estrus after TAI, as compared to the utilization of MGA. Previously, open animals that did not show estrus was considered problematic, so the use of ECP was helpful. However, there seems to be little benefit to resynchronizing the follicle wave when a used CIDR is inserted on Day 13 after fixed-time AI.

Breeding studies in the field revealed that pregnancy rate to fixed-time AI did not differ between cattle given a new or a once-used CIDR, but pregnancy rate was lower in cattle synchronized with a twice-used CIDR; the insertion of two twice-used CIDR did not improve pregnancy rates. The addition of an injection of progesterone to the estradiol treatment at CIDR insertion did not enhance pregnancy rate to fixed-time AI, but there was a suggested benefit when twice-used CIDR were used. These findings are important in that reuse of CIDR can substantially reduce costs; however, sterility is of the utmost importance. We have now shown that used CIDR can be successfully autoclaved. The apparent success of estradiol without an injection of progesterone means that protocols can be simplified and costs (albeit small) can be reduced.

Gonadotropinh releasing hormone- (GnRH) based (Ovsynch or Cosynch) fixed-time AI protocols have been successfully used in mature cattle, but pregnancy rates in heifers have been substantially reduced. We hypothesized that heifers would respond more predictably to an injection of GnRH if they were all at the same stage of follicle development at the time of treatment. We proposed to presynchronize heifers with an injection of PGF 11 days before initiating a GnRH-based program. Presynchronization with PGF prior to a Cosynch protocol reduced the proportion of heifers in estrus before fixed-time AI, suggesting that this approach could be useful in the successful application of Ovsynch or Cosynch programs in heifers. However, pregnancy rate to fixed-time AI was not improved by presynchronization. This must be investigated further in order to determine how presynchronization can be best used to increase pregnancy rates in heifers. In addition, diameter of the preovulatory follicle in this study tended to positively affect pregnancy rate, regardless of treatment used, leading to the conclusion that

studies on factors affecting ovulatory follicle size will be important in improving pregnancy rates following estrus synchronization and AI or fixed-time AI.

These same protocols were applied to the synchronization of embryo transfer recipients, eliminating the need for estrus detection. Both estradiol- and GnRH-based synchronization protocols resulted in acceptable rates of recipient utilization and pregnancy rates following Direct Transfer of frozen-thawed embryos. This will be of immense value because estrus detection, and its inefficiencies is one the major cost factors associated with bovine embryo transfer. Furthermore, more than 95% of recipients synchronized in this study received an embryo, whereas many other reports in the literature have shown that approximately 50% of recipients synchronized normally receive an embryo. This is another substantial cost benefit to the use of fixed-time AI protocols to synchronize embryo transfer recipients. In this study, both CL diameter (a reflection of ovulatory follicle size) and the presence of a central luteal cavity at embryo transfer, significantly affected pregnancy rate, providing additional evidence that preovulatory follicle size can have an influence on the establishment of pregnancy. Additional studies on the use of these protocols in recipients would appear to be warranted.

Overall, results generated over the past year would have to be considered very positive. Protocols that synchronize both follicular wave emergence and ovulation permitted the use of fixed-time AI without the necessity for estrus detection. Treatments initiated near the end of the cycle and the use of GnRH in heifers continue to provide challenges. However, presynchronization with PGF did offer some benefits in heifers; this must be further investigated. A used CIDR was shown to be very beneficial in the resynchronization of "repeats" following fixed-time AI, and the commercial product, ECP, did result in an increased number of open animals showing behavioural estrus. Studies with fixed-time AI and embryo transfer strongly suggest that size of the ovulatory follicle will influence pregnancy rates providing additional challenges that must be investigated.

Technical Report

This project was funded through the Saskatchewan Agriculture Development Fund – Strategic Research Program (SRP Transition) with "in-kind" support from the pharmaceutical industries. Additional (matching) funds were requested from the Alberta Agriculture Research Institute but were not granted. This reports covers a period in 2003-2004 over which Transitional Funds were provided. Our collaboration with Dr. John Kastelic, Agriculture and Agri-Food Canada (Lethbridge), and more recently, Dr. Julie Small, Agriculture and Agri-Food Canada (Brandon), has been very productive. The valuable contributions of post-graduate student, Marcos Colazo, are gratefully acknowledged. These projects will form part of his thesis. The following is a summary of the experiments conducted in 2003-2004.

Detailed Ultrasound Studies of Ovary-intact Heifers

Experiment 1 - Synchronization of follicular wave emergence with estradiol valerate

Objective: To investigate the effect of dose of estradiol valerate (EV) on ovarian follicular growth profiles and intervals to follicular wave emergence and, following CIDR removal, estrus and ovulation in beef cows.

Methods: On Day 0, 43 non-lactating, crossbred beef cows and at random stages of the estrous cycle, received a CIDR and were randomly allocated to one of four groups to receive no further treatment (Control; n = 10), or an injection of 1 mg (n = 11), 2 mg (n = 10), or 5 mg (n = 12) im of EV in 2 mL canola oil. On Day 7, CIDR were removed and cows received Estrumate (PGF). Ovaries were examined daily by transrectal ultrasonography until 48 h after CIDR removal to detect ovarian follicle growth profiles, and twice daily thereafter to detect ovulation.

Results: Results are shown in Table 1. Follicular wave emergence occurred within 7 d in 7/10 (70%) Control cows and 31/32 (97%; P<0.04) EV-treated cows (one cow in late diestrus at the time of treatment did not respond to 1 mg EV). Mean (± SD) interval from treatment to wave emergence was longer (P<0.03) in cows treated with 5 mg EV (4.8 ± 1.2 d) than in those treated with 1 mg (3.2 ± 0.9 d) or 2 mg EV (3.4 ± 0.8 d), while Control cows were intermediate (3.8 ± 2.0 d). Although follicular wave emergence tended (P<0.09) to be more synchronous in cows receiving EV, intervals from CIDR removal to estrus (P=0.7) and ovulation (P=0.8) did not differ from Controls. Diameter of the dominant follicle at CIDR removal was less variable (P<0.01) in the 2 and 5 mg EV groups than in the Control group and intermediate in the 1 mg EV group.

Table 1. Mean (± SD) intervals from treatment with 0, 1, 2 or 5 mg estradiol valerate at CIDR insertion to follicular wave emergence (FWE), and from CIDR removal to estrus and ovulation.

	Dose of estradiol valerate (mg)			
	0	1	2	5
Interval (d) to FWE				
No. cows	10	11	10	10
Mean	2.2[a]	2.5[a]	3.4[a]	4.8[b]
SD	3.3[x]	2.3[x]	0.8[y]	1.2[y]
Range	-4 to 6	-4 to 4	2 to 5	3 to 7
CIDR removal to:				
Estrus				
No. cows	9	11	10	8
Mean	58.0	53.5	61.2	59.3
SD	19.4	11.2	14.1	17.1
Range	36 to 96	36 to 72	48 to 84	48 to 96
Ovulation				
No. cows	10	11	10	10
Mean	86.4	85.1	91.2	90.0
SD	18.6	12.5	14.1	22.1
Range	60 to 120	72 to 108	72 to 120	72 to 132

[ab] Means within the row differ (P=0.045)

[xy] Standard deviations within the row differ (P<0.0008)

In summary, dose of EV affected follicular dynamics, interval to and synchrony of follicular wave emergence, and dominant follicle diameter at CIDR removal and just prior to ovulation in CIDR-treated cows. However, interval from CIDR removal to estrus and ovulation was not affected by treatment. Results suggest that a dose of 2 mg EV may be most efficacious in synchronizing follicular wave emergence in CIDR-treated cows.

Experiment 2 - Effect of estradiol treatment during late stages of the estrous cycle.

Objective: To characterize ovarian follicular dynamics in beef cows treated with a CIDR and an injection of estradiol-17β (E2), with or without progesterone (P4), late in the estrous cycle.

Methods: Non-lactating, crossbred beef cows (n = 36) received a CIDR 16 to 18 d after ovulation (Day 0) and were randomly allocated to one of three treatment groups: no further treatment (Control, n = 12), an injection of 5 mg E2 (E2, n = 12), or 5 mg E2 plus 100 mg P4 (E2P4, n = 12). On Day 7, CIDR were removed and cows received PGF. Ovaries were examined daily by transrectal ultrasonography to detect ovarian follicle growth profiles, and determine the time of ovulation.

Results: Thirteen cows ovulated within 3 d of treatment (50% of E2- and E2P4-treated cows and 8.3% of Control cows; P=0.05); cows that ovulated had smaller CL diameters (15.2 ± 1.7 vs 17.7 ± 2.7 mm; P<0.004) and lower progesterone concentrations (0.4 ± 0.2 vs 2.1 ± 2.2 ng/mL; P<0.001) at the time of treatment. Follicular wave emergence occurred within 7 d in 4/12 Control cows, 10/12 E2-treated cows, and 10/12 E2P4-treated cows (P<0.01). Although the interval from treatment to wave emergence did not differ among treatments (P=0.8; overall, 3.4 ± 1.5 d), follicular wave emergence was more synchronous (P<0.004) in the E2 group than in the Control or E2P4 groups. Dominant follicle diameter was larger (P<0.02) in the Control group (15.9 ± 5.5 mm) than in the E2 (11.9 ± 1.8 mm) or E2P4 (11.5 ± 3.4 mm) groups at CIDR removal, but dominant follicle diameter was less variable (P<0.003) in the E2 group than in the other two groups. Three cows did not ovulate after CIDR removal; two in the Control group and one in the E2P4 group. Interval to ovulation was shorter (P<0.05) in the Control group (70.8 ± 10.5 h) than in the E2 (87.0 ± 9.0 h) or E2P4 (86.2 ± 7.2 h) groups, and the intervals to ovulation in cows that ovulated following treatment (91.0 ± 8.0 h) was longer (P<0.001) than those that did not (76.6 ± 9.6 h). Results are shown in Table 2.

Table 2. Numbers of cows with a new follicular wave emergence (FWE) following treatment with a CIDR and estradiol-17β (E2) oe E2 and progesterone (E2P4) 16 to 18 d after ovulation (Day 0), and mean (± SD) intervals to estrus and ovulation following CIDR removal.

	Treatments on Days 16 - 18		
	Control	E2	E2P4
No. cows treated	12	12	12
No. with a new FWE (%)	4 (33.3)[a]	10 (83.3)[b]	10 (83.3)[b]
Interval (d) from treatment to FWE			
n	4	10	10
Mean	3.8	3.2	3.4
SD	2.8[c]	0.6[d]	1.7[c]
Range	1 to 7	2 to 4	2 to 8
Interval (h) from CIDR removal			
To Estrus			
n	10	12	11
Mean	43.8[e]	57.0[f]	57.8[f]
SD	9.0	8.3	8.2
Range	30 to 60	48 to 72	48 to 72
To ovulation			
n	10	12	11
Mean	70.8[g]	87.0[h]	86.2[h]
SD	10.5	9.0	7.2
Range	60 to 96	72 to 108	72 to 96

[ab] Percentages within a row differ; P<0.01
[cd] Standard deviations within a row differ; P<0.004
[ef] Means within a row differ; P<0.0009
[gh] Means within a row differ; P<0.0003

In summary, treatment of cows with an estradiol-progesterone protocol late in the estrous cycle resulted in ovulation (50.0%), atresia (33.3%) or persistence (16.6%) of the dominant follicle present at that time. As length of follicular dominance and timing of ovulation were affected, fertility could be impaired following AI. This is an important impediment to estrus synchronization that must be overcome.

<u>Experiment 3</u> – Synchronization follicular wave emergence for re-synchronization of nonpregnant (repeats) animals

Objective: To investigate the effects of progesterone, estradiol cypionate (ECP), or GnRH on follicle dynamics, estrus and interval to ovulation in cattle treated 13 d after ovulation with a used CIDR.

Field Experiments

Experiment 1 - Effects of new or used CIDR and ECP treatment, with or without progesterone, on pregnancy rate to timed AI in beef heifers.

Objective: To compare pregnancy rates following fixed-time AI in beef cattle given a new or previously used CIDR and injections of estradiol cypionate (ECP), with or without a commercial source of progesterone, to synchronize follicular wave emergence.

Methods: Heifers (n = 616) received a new or once-used CIDR for 9 d and 1 mg estradiol cypionate (ECP), with or without 100 mg of a commercial progesterone preparation (CP4), at CIDR insertion. Heifers were treated with PGF at CIDR removal and 0.5 mg ECP 24 h later, with fixed-time AI 56 to 60 h after CIDR removal. In a second experiment, heifers (n = 56) and lactating cows (n = 307), at two different locations, received a once- or twice-used CIDR and an injection of 1 mg estradiol benzoate (EB), with or without 100 mg progesterone, at CIDR insertion. Cattle received PGF at CIDR removal on Day 7 and 1 mg EB 24 h later, with fixed-time AI 52 to 56 h after CIDR removal.

Results: In the first experiment, pregnancy rate was not affected by either the addition of progesterone (P=0.42; 48.8 vs 45.6% for ECP and ECP+CP4, respectively), or the number of CIDR uses (P=0.59; 48.3 vs 46.2% for new and once-used CIDR, respectively). In the second experiment, pregnancy rate differed for location (P<0.002; 46.1 vs 61.6% for Locations A and B, respectively), parity (P<0.04; 67.9 vs 53.1% in heifers and cows, respectively) and numbers of times the CIDR had been used (P<0.03; 61.2 vs 49.7% for once- and twice-used CIDR, respectively). However, the addition of progesterone to the injection of EB at CIDR insertion did not affect pregnancy rate (P=0.6). Results are shown in Table 4.

Table 4. Pregnancy rates after fixed-time AI in beef heifers that received a new, a once-used or twice-used CIDR plus an injection of estradiol with (+CP4) or without (-CP4) commercial progesterone at the time of CIDR insertion (first and second experiments).

	CIDR					
	New		Once-used		Twice-used	
	+CP4	-CP4	+CP4	-CP4	+CP4	-CP4
First experiment						
Heifers pregnant (n)	70/149	75/151	71/160	75/156		
Pregnancy rate (%)	46.9	49.6	44.3	48.0		
Second experiment						
Cattle pregnant			60/93	53/88	46/90	42/92
Pregnancy rate (%)			64.5[c]	60.2[c]	51.1[cd]	45.6[d]

[cd] Within a row, percentages without a common superscript differ (P<0.05)

Methods: Non-lactating beef cows received a once-used CIDR 13 d after ovulation (Day 0) and were randomly placed in one of four groups to receive no further treatment (Control; n=10), 150 mg of progesterone (P4; n=11), 100 μg GnRH (n=11) or 1 mg ECP (n=11) at that time. On Day 7, CIDR were removed from all cows and half received 0.5 mg ECP. Ultrasonography was performed to determine follicle growth, and ovulation.

Results: The dominant follicle present at the time of CIDR insertion was the ovulatory follicle in 50.0, 54.5, 18.2, and 36.4% of cows in Control, P4, GnRH, and ECP groups, respectively (P=0.3). Follicle wave emergence following treatment was more synchronous (P<0.0001), and diameter of the dominant follicle at CIDR removal was less variable (P<0.02) in the GnRH group than in the other three groups. Intervals from CIDR removal to estrus and ovulation were not affected by treatments at CIDR insertion (P=0.2). However, 10/43 cows were not seen in estrus; 13.6 % of those receiving ECP and 33.3% of those not receiving ECP at the time of CIDR removal (P<0.1). Although the interval to ovulation tended to be affected (P<0.07) by administration of ECP at CIDR removal (68.7 ± 9.2 vs 75.0 ± 12.2 h for ECP and Control groups, respectively), one cow in the Control group did not ovulate by Day 12. Results are shown in Table 3.

Table 3. Mean (± SD) intervals from treatment to follicular wave emergence (FWE) and mean (± SD) diameters of dominant follicle prior to ovulation in beef cows treated with CIDR alone (Control), 1 mg estradiol cypionate (ECP), 100 μg GnRH (GnRH) or 150 mg progesterone (P4). At CIDR removal (7 d after insertion), half of the cows received 0.5 mg ECP.

	Control	ECP	GnRH	P4
No. cows	10	11	11	11
Interval (d) treatment to FWE				
Mean	0.2	0.7	0.8	-0.2
SD[1]	3.7	4.1	0.8	3.3
Range	-5 to 6	-6 to 5	-1 to 2	-6 to 5
Diameter (mm) dominant follicle				
Prior ovulation				
Mean	17.7[a]	15.3[b]	15.6[b]	16.4[ab]
SD	2.6	2.0	1.4	2.5
Range	14 to 21	13 to 20	14 to 18	13 to 21

[ab] Means within the row differ (P=0.02)
[1] Standard deviations within the row differ (P<0.0001)

In summary, GnRH at the time of used CIDR insertion synchronized follicular wave emergence without affecting estrous cycle length. Administration of ECP at CIDR removal tended to increase the numbers of cows showing behavioural estrus suggesting that this may be useful in synchronizing return to estrus following TAI. Neither ECP nor P4 at the time of used CIDR insertion synchronized follicle wave emergence in this study. It was surprising trhat GnRH did not affect estrous cycle length; this must be investigated further.

In a third experiment, heifers (n = 187) received a new, a once-used, a twice-used or two twice-used CIDRs for 7 d and 2 mg EB plus 50 mg of CP4 at the time of CIDR insertion. Heifers were treated with PGF at CIDR removal and 1 mg EB 24 h later, with fixed-time AI 54 to 58 h after CIDR removal. Pregnancy rate was not affected by treatments (P=0.28; 57.5, 63.8, 52.1, 52.1% for a new, a once-used, a twice used or two twice-used CIDR inserts, respectively).

In summary, pregnancy rate to fixed-time AI did not differ between cattle given a new or once-used CIDR, but pregnancy rate was lower in cattle receiving a twice-used CIDR; the insertion of two twice-used CIDR did not improve pregnancy rates. The addition of an injection of progesterone to the estradiol treatment at CIDR insertion did not enhance pregnancy rate to TAI. These are important findings from the perspective of cost and user=friendliness. However, we must ensure that used CIDR are adequately sterilized; we have now shown that CIDR can be effectively autoclaved.

Experiment 2 – Presynchronization and timed AI in Ovsynch-type protocols

Objective: To investigate the effect of presynchronization with PGF prior to a GnRH-based, Cosynch protocol on estrus synchrony, CL and preovulatory follicle diameters and pregnancy rate following fixed-time AI in beef heifers.

Methods: Cycling beef heifers (n = 148) were treated with 100 μg GnRH on Day 0, PGF on Day 7, and GnRH concurrent with fixed-time AI on Day 9 (54 h after PGF). Half of the heifers (Control) received the first GnRH treatment at random stages of the estrous cycle, while the other half (Presynch) received two injections of PGF 11 d apart, with the first injection of GnRH 11 d after the second injection of PGF. Estrus detection was done between the first GnRH and 12 h after PGF, and heifers detected in estrus were inseminated (and considered nonpregnant to fixed-time AI), while all other heifers were fixed-time inseminated.
Results: The numbers of heifers in estrus early (after first GnRH and before fixed-time AI) was higher in the Control group than in the Presynch group (18/74 vs 2/74, respectively; P<0.0001). Although the diameter of the preovulatory follicle was more variable (P<0.004) in Control (5 to 19 mm) than Presynch (8 to 17 mm) heifers, pregnancy rate to fixed-time AI did not differ (P=0.4; 29.7 vs 36.5%, respectively). Overall pregnancy rates were 45.9 and 37.8% for Control and Presynch groups, respectively (P=0.3). Pregnancy rate tended (P<0.08) to be affected by diameter of the preovulatory follicle at the time of fixed-time AI (0, 23.1, 45.7, 41.4, and 60.0% pregnant for diameters of <9, 9-11, 12-14, 15-17, and >17 mm, respectively). Regardless of treatment, diameter of the preovulatory dominant follicle (P<0.02) and CL (P<0.03) 7 d after fixed-time AI was smaller, and CL diameter was more variable (P<0.004), in open than in pregnant heifers (12.7 ± 2.6 vs 13.8 ± 2.1 mm, and 16.5 ± 4.4 vs 18.0 ± 3.0 mm, respectively). Results are shown in Table 5.

Table 5. Proportion (%) of heifers detected in estrus and inseminated before TAI and pregnancy rates following AI, TAI and exposure to bulls. Results are shown in Table 5.

	Presynch	Control
Number heifers (n)	74	74
Estrus and AI - Days 0 to 8 (n)	2.7^{a} (2)	24.3^{b} (18)
AI pregnancy (%)[1]	50.0	66.6
Timed-AI (n)	97.3^{a} (72)	75.7^{b} (56)
TAI pregnancy (%)[2]	37.5	29.7
Total pregnant - Day 36 (%)	37.8	45.9
Cumulative pregnancy - Day 63 (%)[3]	83.8	85.1

^{ab} proportions in rows with different letters differ ($P<0.05$)

1) Heifers inseminated early were considered nonpregnant to TAI

2) Pregnancy diagnosis was done 27 d after TAI (Day 36 of the treatment schedule)

3) Cumulative following AI, TAI and bull breeding

In summary, presynchronization with PGF prior to a Cosynch protocol reduced the proportion of heifers in estrus before fixed-time AI, suggesting that this approach may be useful in the successful application of Ovsynch or Cosynch programs in heifers. However, pregnancy rate to fixed-time AI did not differ between groups in this study. Diameter of the preovulatory follicle tended to positively affect pregnancy rate, regardless of treatment. The presynchronization treatment shows promise, but we have not yet determine the most appropriate way to use it.

Experiment 3 – Resynchronization with MGA or a used-CIDR plus GnRH and ECP

Objective: To investigate the effects of a used CIDR, MGA, estradiol cypionate (ECP), and GnRH on estrus, conception and pregnancy rates in beef heifers resynchronized following fixed-time AI.

Methods: Beef heifers were assigned to two different resynchronization experiments starting 13 d after fixed-time AI. In the first experiment, heifers (n=675) were fed MGA (0.4 mg/head/day) for 6 d and allocated in two groups to receive no further treatment (Control) or 100 μg GnRH at that time (Day 0). On Day 7, half of the heifers received 0.5 mg ECP. In the second experiment, heifers (n=317) were fed MGA as in the first experiment or given a once-used CIDR insert for 7 d. On Day 7, CIDR were removed and 0.5 mg ECP was given to half of the heifers. In both experiments, visual observation for estrus was done 3 times daily (for 40 min each) from Days 8 to 12 and heifers were inseminated 6 to 12 h after the onset of estrus. Pregnancy diagnosis was done approximately 55 d after AI by transrectal ultrasonography.

Results: Overall, pregnancy rate to fixed-time AI was 38.1% and no treatments in either experiment adversely affected pregnancy rate to fixed-time AI; 419 and 195 heifers, respectively, were not pregnant to TAI. In the first experiment, estrus rate tended (P<0.1) to be higher in the group treated with ECP on Day 7 (69.6 vs 62.7%), while treatment with GnRH on Day 0 had no effect on estrus rate (P=0.5). Conception (47.3 %) and pregnancy (31.3%) rates were not affected by treatment. In the second experiment, there was no effect of treatment on estrus rate (P=0.4; overall, 73.8%). Moreover, treatment with ECP on Day 7 had no effect on conception (P=0.4) or pregnancy (P=0.8) rate. However, heifers treated with CIDR inserts tended (P<0.1) to have higher conception rates (68.8 vs 56.7%) and pregnancy rate was higher (P<0.04; 54.1 vs 39.2%) than those fed MGA.

In summary, fertility was higher when a used CIDR was used to resynchronize return to estrus after fixed-time AI than when MGA was used. Administration of 0.5 mg ECP on Day 7 tended to increase the number of open heifers showing estrus without affecting fertility to fixed AI. Although detailed ultrasound studies (Experiment3) showed that treatment with GnRH on Day 13 (day of used CIDR insertion) effectively synchronized follicle wave emergence, treatment with GnRH at the time of CIDR insertion in this field study did not affect any of the parameters examined. This requires additional study.

<u>Experiment 4</u> – Embryo transfer without estrus detection.

Objective: To compare two synchronization protocols for recipients, without the need for estrus detection, using products commercially available in North America.

Methods: On Day 0 (first day of the experiment), lactating beef cows (n = 70) with a body condition score between 1.5 and 3.5 (1 to 5 scale) were scanned ultrasonically for reproductive tract abnormalities and each received a CIDR; 36 cows (ECP group) were concurrently given injections of 1 mg ECP and 50 mg commercial progesterone. On Day 1, the other 34 cows (GnRH group) were injected with 100 μg of GnRH. At the time of CIDR removal (Day 8), cows were scanned ultrasonically and given PGF. Cows in the ECP or GnRH groups were subsequently given injections of 0.5 mg ECP and 100 μg GnRH on Days 9 and 10, respectively. On Day 17, CL size was measured ultrasonically; cows with a CL > 10 mm in diameter received a frozen-thawed embryo by nonsurgical, Direct Transfer (33/36, 91.6% and 32/34, 94.1% for cows in the ECP and GnRH groups, respectively).

Results: There was no difference (P<0.5) between the ECP- and GnRH-treated groups for diameter of the CL (22.7 ± 6.3 and 21.7 ± 5.3 mm), luteal area (302 ± 98 and 296 ± 98 mm^2) on the day of embryo transfer, or pregnancy rate 68 d after transfer (14/33, 42.4% and 13/32, 40.6%). However, pregnancy rate was affected by both CL diameter (≤ 18 mm, 3/17, 17.6% vs > 18 mm, 24/48, 50.0%; P<0.02) and the presence of a central luteal cavity at embryo transfer (21/43, 48.8% vs 6/22, 27.2%, for cows with and without a cavity, respectively; P<0.05).

In summary, both protocols resulted in high rates of recipient utilization and acceptable pregnancy rates to Direct Transfer of previously frozen embryos. However, both CL diameter and the presence of a central luteal cavity at transfer significantly affected pregnancy rate. This provides additional support for the notion that ovulatory follicle size affects pregnancy rates (to

fixed-time AI – Experiment 2 and following embryo transfer – Experiment 4). Follicle size and maturity may be next important area to be investigated in order to improve pregnancy rates following the use of assisted reproductive technologies in cattle, and may be a means of improving pregnancy rates following traditional estrus detection and AI.

Although results generated over the past year would have to be considered very positive, there are also areas that require further investigation. Results confirm that protocols which synchronize both follicular wave emergence and ovulation will permit the use of fixed-time AI, without the necessity for estrus detection, while yielding acceptable pregnancy rates. Estradiol valerate at the appropriate dose was very effective in synchronizing follicle wave emergence, and a once used-CIDR was as efficacious as a new CIDR in a fixed-time AI protocol; challenges now arise concerning sterilization after use. The addition of progesterone treatment at the time of estradiol treatment did not improve pregnancy rates to fixed-time AI. However, estradiol treatments initiated near the end of the cycle and the use of GnRH in heifers continue to provide challenges that must be overcome. Presynchronization with PGF did offer some benefits in heifers by preventing animals from coming into estrus early, but pregnancy rates to fixed-time AI were not improved; this must be further investigated. A used CIDR was shown to be very beneficial in the resynchronization of "repeats" following fixed-time AI, and the commercial product, ECP, resulted in an increased number of open animals showing behavioural estrus. On the other hand, the use of either GnRH, ECP or progesterone to synchronize follicle wave emergence was not beneficial in resynchronization protocols; a used CIDR was more effective than MGA in resynchronizing repeats. Fixed-time AI protocols were shown to be useful in the synchronization of embryo transfer recipients, eliminating the need for estrus detection. Studies with both fixed-time AI and embryo transfer strongly suggest that size of the ovulatory follicle (and ultimately the size and function of the resulting CL) will influence pregnancy rates. Subsequent studies must be directed toward factors that affect pregnancy rates.

Publications since last ADF-Strategic Grant Report

Martínez M.F., Kastelic J.P., and Mapletoft R.J. 2004. The Use Of Estradiol and/or GnRH in a Two-dose PGF Protocol for Breeding Management of Beef Heifers. Theriogenology, (In press).

Small J., Colazo M., Ambrose D., Mapletoft R., Reeb J., and Kastelic J. 2004. Pregnancy Rate Following Transfer of in vitro- and in vivo-produced Bovine Embryos to LH-treated Recipients. Reproduction, Fertility and Development, 16, 213.

Mapletoft R.J., Colazo M.G., Small J.A., Ward D.R., and Kastelic J.P. 2004. Effect of Dose of Estradiol Valerate on Ovarian Follicular Dynamics in CIDR-treated Beef Cows. Reproduction, Fertility and Development, 16, 130.

Kastelic J.P., Colazo M.G., Small J.A., Ward D.R., and Mapletoft R.J. 2004. Ovarian Follicular Dynamics in Cows Treated with a CIDR, Estradiol and Progesterone Late in the Estrous Cycle. Reproduction, Fertility and Development, 16, 129.

Colazo M.G., Small J.A., Ward D.R., Erickson N.E., Kastelic J.P., and Mapletoft R.J. 2004. The Effect of Presynchronization on Pregnancy Rate to Fixed-time AI in Beef Heifers Subjected to a Cosynch Protocol. Reproduction, Fertility and Development, 16, 128.

Colazo M.G., Kastelic J.P., Martinez M.F., Whittaker P.R., Wilde R., Ambrose J.D., Corbett R. and Mapletoft R.J. 2004. Fertility Following Fixed-time AI in CIDR-treated Beef Heifers given GnRH or estradiol Cypionate and Fed Diets Supplemented with Flax Seed or Sunflower Seed. Theriogenology, 61, 1115-1124.

Colazo M.G., Kastelic J.P., Whittaker P.R., Gavaga Q.A., Wilde R., Mapletoft R.J. 2004. Fertility in beef cattle given a new or previously used CIDR insert and estradiol, with or without progesterone. Animal reproduction Science, 81, 25-34.

Colazo, Marcos German, Kastelic, John Patrick, Mapletoft, Reuben John., 2003. Effects of Estradiol Cypionate (ECP) on Ovarian Follicular Dynamics, Synchrony of Ovulation and Fertility in CIDR-based, Fixed-time AI Programs in Beef Heifers. Theriogenology, 60, 855-865.

Martinez, M.F., Mapletoft, R.J., Kastelic, J.P. and Carruthers, T.D., 2003. The Effects of Three Gonadorelin Products on Luteinizing Hormone Release, Ovulation, and Follicular Wave Emergence in Cattle. Canadian Veterinary Journal, 44, 125-131.

Colazo, M.G., Whittaker, P.R., Kastelic, J.P. and Mapletoft, R.J. 2003. CIDR-based protocols for synchronizing bovine embryo transfer recipients without estrus detection. Journal of Animal Science, 81 (Suppl. 1)/Journal of Dairy Science, 86 (Suppl. 1), 183.

Colazo, M.G., Gavaga, Q.A., Kastelic, J.P. and Mapletoft, R.J., 2003. The Effects of Estradiol Cypionate (ECP) at CIDR Removal and Interval to Fixed-time AI on Pregnancy Rates in Beef Cattle. Theriogenology, 59, 215.

Kastelic, J.P., Colazo, M.G., Martinez, M.F. and Mapletoft, R.J., 2003. Effects of a CIDR or MGA, Reproductive Status and Estradiol, GnRH or pLH Treatment on Pregnancy Rate to Fixed-time AI in Beef Heifers. Theriogenology, 59, 223.

Martínez, M.F., Colazo, M.G., Kastelic, J.P. and Mapletoft, R.J., 2003. Effects of Estradiol and Progesterone on Plasma Steroid and Gonadotrohin Concentrations in CIDR-treated Ovariectomized Cows. Theriogenology, 59, 224.

Mapletoft, R.J., Colazo, M.G., Martinez, M.F. and Kastelic, K.P., 2003. Esteres de Estrogeno para la Sincronizacion de la Emergencia de la Onda Follicular y la Ovulacion en Animales Tratados con Dispositivos con Progesterona. V Simposio Internacional de Reproduccion Animal. Heuta Grande, Cordoba, Argentina, pages 55-70.

Kastelic, K.P. and Mapletoft, R.J., 2003. Causas no Infecciosas de Muerte Embrionaria en Ganado Bovino. V Simposio Internacional de Reproduccion Animal. Heuta Grande, Cordoba, Argentina, pages 149-160.

Barth, A.D., Thundathil, J.C. and Mapletoft, R.J., 2003. Importancia de la Calidad Seminal y el Uso de FIV para el Estudio de Defectos Espermaticos. V Simposio Internacional de Reproduccion Animal. Heuta Grande, Cordoba, Argentina, pages 205-222S.

Mapletoft, R.J., Martinez, M.F., Colazo, M.G. and Kastelic, J.P., 2003. The Use of Controlled Internal Drug Release Devices for the Regulation of Bovine Reproduction. Journal of Animal Science, 81(E. Suppl. 2):E28–E36.

Personnel over 2003-2004
Reuben J. Mapletoft – Investigator
John P. Kastelic – Co-investigator (AAFC, Lethbridge)
Julie Small – Collaborator (AAFC, Brandon)
Marcos Colazo – Graduate student (Paid in part off this grant)
Nathan Erickson – Summer student (Paid in part of this grant)

Equipment
None purchased.

Project Developed Materials or Project Photos - None.

Acknowledgement
Saskatchewan Department of Agriculture, Food and Revitalization has been acknowledged on all publications that have arisen out of this project.